Receptor clustering: **Activate to accumulate?**

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Postsynaptic receptor clustering is thought to be of critical importance in central neurotransmission. Recent work suggests that the formation and size of such clusters may depend on synaptic activity, although that dependence appears to vary according to the type of receptor that mediates the postsynaptic response.

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Information transfer in the central nervous system (CNS) occurs between neurons connected by chemical synapses. These highly specialized structures consist of a presynaptic element, which releases neurotransmitter molecules, and a postsynaptic structure, which detects and responds to this release. Neurons maintain the fidelity of neurotransmission by accumulating, or 'clustering', neurotransmitter receptors at sites precisely opposing presynaptic transmitter release. The mechanism by which receptors are selectively targeted to, and maintained at, central synapses is the subject of intense research. Recent studies have provided new insights into the part synaptic activity itself plays in regulating the formation of postsynaptic receptor clustering.

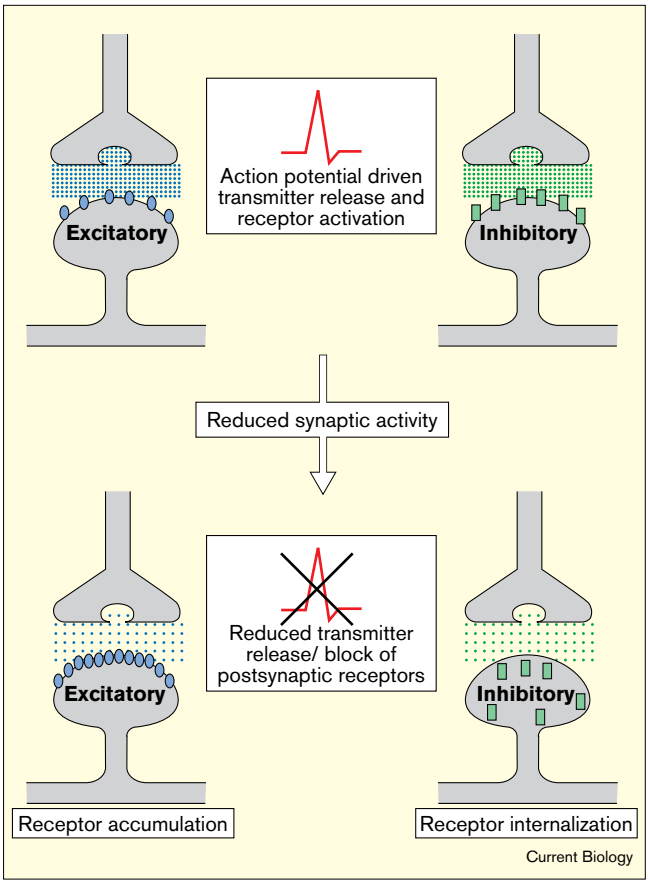
At the neuromuscular junction, a model system for studying synaptogenesis, a number of factors have been shown to be crucial for the clustering of nicotinic acetylcholine receptors. For example, agrin, a protein released from the presynaptic nerve terminal, is needed to induce aggregation of receptors on muscle cells, while a postsynaptic protein, rapsyn, is also required for receptor clustering. It has long been thought that clustering of receptors at CNS synapses also requires signals from the presynaptic element, in addition to a postsynaptic apparatus, to regulate synthesis and targeting of receptors. But the nature of these signals, and the identity of postsynaptic proteins involved in the process, have remained elusive.

In the mammalian CNS, neuronal activity is mediated through the actions of excitatory and inhibitory neurotransmitters. Glutamate is the major excitatory transmitter, while γ-aminobutyric acid (GABA) and glycine are the primary inhibitory neurotransmitters. In each neuron, the synthesis, sorting and anchoring of inhibitory and excitatory transmitter receptors occur sequentially in such a way that some synapses accumulate excitatory receptors, whereas others accumulate inhibitory receptors. These clusters of inhibitory and excitatory receptors can occur on adjacent synapses on the same neuron [1], demonstrating the high spatial selectivity with which neurotransmitter receptors are targeted and anchored to CNS synapses. Although it is far from clear how a nascent synapse decides whether it will eventually be excitatory or inhibitory, some of the factors that may be required to stabilize inhibitory and excitatory receptors at their appropriate synapses are beginning to be identified.

Work on neurotransmitter receptor distribution has been greatly facilitated by the recent development of specific fluorescently-labeled antibodies against various receptors. In combination with confocal microscopy, immunofluorescence allows the detection of changes in the distribution of receptors on the cell surface or intracellular compartments. Kirsch and Betz [2] have recently used these methods to investigate the role of receptor activation in the formation of glycine receptor clusters at glycinergic synapses in spinal neurons. Betz and colleagues [3] had previously shown that the postsynaptic protein gephrin is required for glycine receptor clustering. The idea is that one domain of gephrin associates with cytoskeletal elements in clusters, while another region interacts with glycine receptors. During early development and before synapse formation, glycine receptors are diffusely distributed on the cell membrane or in intracellular compartments. During synaptogenesis, however, clusters of gephrin begin to form, and then recruit membrane diffuse glycine receptors and tether them to sites opposing the presynaptic terminal [4].

What causes the gephrin molecules to cluster in the first place? On the basis of their new results, Kirsch and Betz [2] suggest that postsynaptic glycine receptor activation is required. The action of synaptically released glycine on primordial inhibitory synapses is suggested to cause the clustering of first gephrin, and then glycine receptors, at sites that eventually become mature inhibitory synapses. The ability of glycine to act as a trophic factor, in addition to being a neurotransmitter, was discovered by blocking basal glycine receptor activation with strychnine. When glycine receptors were inhibited in this way, clustering of gephrin, and hence of glycine receptors, was found to be disrupted, and the glycine receptors redistributed to intracellular compartments (Figure 1). It was also found that the L-type Ca^{2+}-channel blocker, nifedipine, mimics the action of strychnine, suggesting that Ca^{2+} entry through voltage-dependent Ca^{2+} channels is a downstream trigger of glycine receptor clustering.

Figure 1



Activity-dependent regulation of synaptic clustering of receptors in spinal neurons. The top panel illustrates the situation for synaptic receptor clustering during normal synaptic activity: excitatory receptors are maintained at a relatively low level at postsynaptic sites, and inhibitory receptors also accumulate at the postsynaptic membrane. When synaptic activity, or postsynaptic receptors, are blocked, receptors either become more concentrated at the synapse (AMPA receptors) or are internalized (glycine receptors). See text for details.

To explain their results, Kirsch and Betz [2] invoke an apparent paradox in developmental neurobiology, namely that, at early stages of development, many synapses that use an inhibitory neurotransmitter are actually (functionally) excitatory. This phenomenon arises because, at these early stages, the equilibrium potential for Cl^- ions — the charge carriers through inhibitory receptors — is such that they exit the neuron when the glycine-receptor channel opens, leading to depolarization of the neuron and activation of voltage-dependent Ca^{2+} channels. The cascade of events that must occur after this Ca^{2+} entry is unknown at present. Furthermore, how glycinergic synapses are maintained once they have formed is unclear, although it appears that postsynaptic glycine receptor activation is not required for this, as strychnine has no effect on the clustering of glycine receptors in mature neurons [2].

The synaptic targeting and stabilization of excitatory neurotransmitter receptors is also an area of recent interest. A primary observation is that glutamate receptors interact directly with a number of other synaptic proteins and that these interactions appear to be required for postsynaptic receptor clustering. They are principally mediated by proteins carrying PDZ domains, so-called because they were initially identified in proteins with the abbreviated names PSD-95, Dlg and ZO-1 [5]. Specifically, NMDA-type glutamate receptors have been found to interact, via their carboxyl termini, with the PSD-95 family of proteins which, as their name suggests, are enriched in the postsynaptic density [6]. Similarly, AMPA-type glutamate receptors interact with the PDZ-domain-containing protein GRIP, and disruption of this interaction causes a disruption of AMPA-receptor clustering [7].

Until recently it was unclear if presynaptic signaling through transmitter release also regulates the postsynaptic clustering of glutamate receptors. Although an early report showed that synaptic activity is not required for glutamate receptor clustering [1], recent evidence now suggests that clustering at excitatory synapses is at least modified by activity [8,9]. This modification was initially shown at excitatory hippocampal synapses, where the number of NMDA receptor clusters was increased when these receptors were blocked [8]. Shortly after this report, Turrigiano *et al.* [9] showed that the size of AMPA-receptor-mediated responses to quantal release of glutamate in neocortical neurons was increased following a period of chronic inhibition of AMPA receptors. Furthermore, neuronal responsiveness to exogenous glutamate application was increased after chronic inhibition. This implied a selective, activity-dependent upregulation of AMPA receptor quantity or efficacy at excitatory synapses.

In our laboratory, we have recently extended these observations to show that the activation of AMPA receptors apparently downregulates their clustering in spinal neurons (unpublished results). Specifically, AMPA receptors become more intensely clustered when their basal activity is blocked with an AMPA receptor antagonist (Figure 1). This implies that glutamate normally acts at its receptors to limit the size of their clusters at synapses. As a corollary to the increase in synaptic receptor accumulation, we observed a parallel increase in the responsiveness of spinal neurons to quantal neurotransmitter release and exogenously applied AMPA receptor agonists, similar to that found by Turrigiano *et al.* [9]. Furthermore, we found that, when the activation of inhibitory receptors is blocked pharmacologically, neuronal excitation predominates and AMPA receptor clusters get smaller, resulting in a reduction in neuronal responsiveness.

Activity-dependent changes in synaptic receptor accumulation are likely to have a number of interesting

physiological consequences. For example, it is thought that neurons employ long-term mechanisms to maintain a relatively constant output response, even though they experience shorter-term changes in the amount of input they receive at individual synapses [10]. This is important, as it sets a limit on how active a cell can become as a result of plastic changes arising from, for example, long-term potentiation (LTP), a current cellular model for learning and memory [11]. After one round of plasticity, the neuron can reset its sensitivity and is able to be potentiated once again. The mechanism for this homeostatic adjustment of output is unknown, but clearly one possibility is that neurons might alter their output strength by a dynamic change in the number of receptors at individual synapses.

In addition to long-term synaptic homeostasis, alterations in glutamate receptor accumulation may occur on a more rapid time scale, as suggested by recent work on 'silent synapses' [12,13]. Under certain conditions, synapses that are functionally unresponsive, or 'silent', can be rapidly activated. One theory espouses the notion that these synapses are converted into active ones by the insertion of latent AMPA receptor clusters into the postsynaptic membrane. Recent studies, including some in our laboratory (unpublished results), have shown that silent synapses can be visualized immunocytochemically in hippocampal neurons: they have the presynaptic and postsynaptic molecular features of excitatory synapses but lack AMPA receptors. The proportion of silent synapses was found to be sensitive to the amount of ambient synaptic activity, as chronic blockade of AMPA receptors caused a dramatic decrease in their number. Furthermore, the number of visualized silent synapses decreased with development in culture, perhaps as a result of developmental changes in spontaneous synaptic activity.

The molecular cascades that occur in response to alterations in the amount of excitation a neuron receives are likely to be complicated, and may involve both transitory post-translational modifications and changes in gene expression. The increase in the size of AMPA receptor clusters that we have observed in spinal neurons is accompanied by an increase in their metabolic half-life (unpublished results). Thus, receptors that would normally have been removed from the synapse by receptor recycling are somehow stabilized. This could occur as a result of changes in the phosphorylation state of the receptor itself or of some ancillary protein, or by the upregulation of interacting proteins that stabilize the receptors at synaptic sites. As dynamic alterations in the gain of synapses — arising at least partly through activity-dependent changes in receptor number — are likely to occur extensively in the CNS during plastic neuronal changes and development, delineation of the molecular pathways leading to such changes is clearly an important challenge.

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